UNITED STATES DEPARTMENT OF COMMERCE
Pat nt and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

PD

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/127,738	08/03/98	PONCE DE LEON	F 002076-005

021839 HM12/1222
BURNS DOANE SWECKER & MATHIS
P O BOX 1404
ALEXANDRIA VA 22313-1404

EXAMINER	
STROUP,C	
ART UNIT	**PAPER NUMBER**
1633	6

DATE MAILED: 12/22/99

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trad marks

Office Action Summary	Application No. 09/127,738	Applicant(s) Ponce De Leon et al
	Examiner Stroup, Carrie	Group Art Unit 1633

☐ Responsive to communication(s) filed on _____

☐ This action is **FINAL.**

☐ Since this application is in condition for allowance except for formal matters, **prosecution as to the merits is closed** in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire 3 month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claim

☒ Claim(s) *1-24* is/are pending in the applicat

Of the above, claim(s) _____ is/are withdrawn from consideration

☐ Claim(s) _____ is/are allowed.

☒ Claim(s) *1-24* is/are rejected.

☐ Claim(s) _____ is/are objected to.

☐ Claims _____ are subject to restriction or election requirement.

Application Papers

☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on _____ is ☐ approved ☐ disapproved.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☒ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number) _____ .

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☒ Notice of References Cited, PTO-892

☒ Information Disclosure Statement(s), PTO-1449, Paper No(s). 5

☐ Interview Summary, PTO-413

☐ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

--- SEE OFFICE ACTION ON THE FOLLOWING PAGES ---

DETAILED ACTION

Claim Rejections - 35 USC § 112

1. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

2. Claims 2-5, 12, 13, 15-19, and 22-23 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Applicant's claimed invention is to a EG cell derived from the culturing of an Gallinacea avian PGC in : leukemia inhibitory factor, basic fibroblast growth factor, stem cell factor, and insulin-like growth factor in amounts sufficient to maintain said PGC's for prolonged periods in tissue culture, and wherein said EG cell is transfected or transformed with a desired nucleic acid sequence, such as a therapeutic protein (claims 12 and 13); and an improved method for producing chimeric avians comprising isolating PGC's from an avian, maintaining said PGC's in a tissue culture medium containing at least the following growth factors: LIF, bFGF, SCF, and IGF for a sufficient time to produce EG cells, transfecting said EG cells with a desired nucleic acid sequence, such as that which encodes a therapeutic polypeptide, prior to transferral into a recipient avian embryo, then selecting for chimeric avians which have the desired PGC phenotype, and purifying said therapeutic polypeptide from the eggs, systemic circulating system, or body fluids or tissues of chimeric avians produced (claims 17-19 and 23).

The specification fails to provide an enabling disclosure for the species from which the growth factors were derived and the minimal amounts of growth factors to be.0625 U/ul of LIF, .25 pg/ul of bFGF, .5625 pg/ul of IGF, and 4.0 pg/ul of SCF, and the maximal amount to be 3-100X of said concentrations (claims 2,3). The specification does not identify what species the growth factors utilized within the culture were derived from, such as human or avian LIF. Chang et al (Cell Biology International, 1995, 19(2): 143-149) teach that , for example, human SCF had no proliferative effect on chick PGCs and further states that "species specificity of chick and mammalian SCFs may have species specific effects on PGCs in culture" (pg 148, col 1, para 2). Therefore, one of skill in the art would be required to practice undue experimentation to utilize the claimed culture medium on any avian PGC's because of the failure to disclose the species from which the exemplified growth factors were derived. Furthermore, the specification discloses that the initial concentrations were .0625 U/ul of LIF, .25 pg/ul of bFGF, .5625 pg/ul of IGF, and 4.0 pg/ul of SCF, but that medium changes were carried out every other day by removing 5ul of medium and adding 5 ul of 2X ne medium. The specification does not disclose, though, the initial volume of the medium, only the concentrations of the growth factors per unit volume. The specification then goes on to assert that the final medium and the net result after an unspecified time of tissue culturing and an unspecified number of changes of medium, contains doubled the concentrations of each of said growth factors, and that the PGCs survive and proliferate better at the highest end of the described growth factor concentrations (pg 30, lines 1-4). The specification, therefore, teaches that the initial concentrations were as per those that were claimed in Claim 2 and 3, but in fact the optimal conditions are twice the initial concentrations. The specification also fails to disclose the use of any concentration above this "optimal", therefore one of skill in the art would be required to practice undue experimentation to utilize a culture medium with growth factor concentrations 3-100X the intial concentrations, and to use a medium with a constant minimal, initial concentrations such that PGCs could be successfully maintained to produce EG cells and a compact multilayered appearance (claim 1).

The specification fails to provide an enabling disclosure for an EG cell encoding a transgene, and an improved method for producing chimeric avians, comprising transfection or transformation of said EG cells with a desired nucleic acid sequence or encoding a therapeutic protein. The specification discloses the teachings from others for the generation of chimeric birds who expressed a transgene, wherein the essential details of the vector composition and method of transfection were the incorporated by references (pg 3, lines 5-pg 6, line 16). It also discloses that "Infection of PGCs with retroviral vectors has also been reported. However, to date, the growth of PGCs in culture for prolonged periods to facilitate selection of transfected PGCs has not been achieved" (pg 6, lines 6-9). Furthermore, the specification teaches that the production of chimeric avians can be accomplished by introducing a desired DNA sequence into the PGCs, via lipofection, transfection, microinjection, wherein the applicants' efforts of introducing a vector containing a green fluorescence reporter gene by lipofection resulted in an average of 1/50 PGCs being transciently transfected, and wherein no stable transfected cell line was developed (pg 20, lines 10-16; pg 43, lines 5-10).

The specification also relies upon general teachings on transfection of a therapeutic polypeptide stating that such includes unspecified growth factors or enzymes, under the regulatory control of sequences operable in avians. The specification fails to disclose specific promoters or regulatory sequences operable in avian cells or the identity of therapeutic polypeptides which would have a utility within avian animals, such as treating a specific disease state. Therefore, it light of the unpredictability in the art of expressing a transgene into an avian PGC in vivo and in vitro, and the failure of the specification to disclose essential teachings for such, ie the vector construct design, it would require undue experimentation by one of skill in the art to transfect or transform EG cells with a nucleic acid sequence such that a therapeutic effect could be achieved, or even stable expression of the transgene *in vivo* or *in vitro*.

The specification also fails to provide an enabling disclosure for a method of purifying a polypeptide from avian eggs, systemic circulatory systems, body fluids, or tissues. It is also a widely accepted fact within the art that protein purification is a difficult and unpredictable process.

> "The purification of any protein involves many steps which often must be practiced in a precise order and under specific conditions of time, temperature, volume, concentration, etc.. These steps are not self-evident, and will vary a great deal from protein to protein. There are literally infinite combinations of possible columns, gradients, gels, precipitants, centrifugations, all with buffers of varying pH, salt, concentrations of same, etc., to chose from. Until a purification has been accomplished, and the protein certainty, there is little guidance as to where one would even begin." (Transcript from *Hearing of the US Patent and Trademark Office*, 10/17/94, San Diego, pg 101)

The specification fails to provide any disclosure, either by general teachings or exemplifications, on the method of purifying any polypeptide from any source. Due to the technical difficulties in the art of protein purification, and the failure of the specification to provide essential details to overcome said difficulties, it would require undue experimentation by one of skill in the art at the time of the invention to purify a polypeptide from avian eggs, systemic circulatory systems, body fluids or tissues.

Lastly, the specification fails to provide an enabling disclosure for the use of a Gallinacea or turkey PGC. Gallinacea genus includes all heavy bodied terrestrial birds including the pheasants, turkeys, grouse, and the common domestic fowl (Webster, pg 477). The specification's teachings and the prior art referenced disclose only the use of chicken avian PGC's, ES's and EG's. The art of producing chimeric animals via culturing and introducing PGC's into the embryo of animals is a highly unpredictable art, wherein the results derived from one species cannot be directly applied to another species even one within the same genus (see Chang et al, supra). The specification also fails to disclose the commercial availability and sequence homology between the different species growth factors, or the proliferation and differentiation of different species PGC's, ie turkey, in the disclosed culture medium. Therefore, it

would require undue experimentation by one of skill in the art to utilize any species other than chicken in the disclosed culture medium such that long-term viability and de-differentiation to EG's could be achieved.

3. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

4. Claims 1-3, 13, and 14 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1-3 are unclear as to the metes and bounds of "sufficient", "minimal", and "maximal" concentrations of culture medium rather than "initial" and "optimal", and the species from which the growth factors were derived (ie human or avian or another?).

Claim 13 is unclear as to the metes and bounds of "therapeutic". Must the protein treat or prevent an avian disease or disorder, or is it, for example, a growth factor that facilitates cell growth and differentiation?

Claim 14 is unclear as to the metes and bounds of "tissue" culture medium. What avian tissues are comprised in this classification?

Claim Rejections - 35 USC § 103

5. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been

obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

6. Claims 1, 5-11, 14, 16, and 20-22 are rejected under 35 U.S.C. 103(a) as being unpatentable over Pain et al (1996) in view of Labosky et al (1994).

Applicant's claimed invention is to a method for culturing avian primordial germ cells (PGS's) and germ cells (EG) comprising the following steps: isolating primordial germ cells from a desired avian and culturing said cells in a culture medium containing at least the following growth factors: leukemia inhibitory factor, basic fibroblast growth factor, stem cell factor, and insulin-like growth factor in amounts sufficient to maintain said PGC's for prolonged periods in tissue culture having a compact multilayer like appearance; and identifying EG cells contained therein.

Pain et al teach the method of culturing avian embryonic stem cells collected from chicken blastoderms at stage X of development, which resulted in a donor derived phenotype thus demonstrating a germ line transmission (pg 2341, col 1, para 4; pg 2344, col 2, para 2). Culture methods included 10ng/ml bFGF, 20 ng/ml h-IGF-1, 1% vol/vol avian-SCF, and 1% vol/vol h-LIF; and wherein a combination of aSCF, bFGF, and mLIF strongly enhanced the number of alkaline-phosphatase-positive colonies (pg 2340, col 1, para 1; pg 2341, col 2, para 3; Fig 2, pg 2342), and where upon transfer into avian embryos develop into PGCs. Pain et al also teach that the use of LIF is necessary for the growth and long-term maintenance of ES cells in culture resulting in cells that were ECMA-7, SSEA-1, and EMA-1 positive for at least 35 passages and more than 160 days (pg 2343, Figure 4, and col 2), and successful germline transmission in Barred Rock black strain chickens (pg 2344, col 2, para 2). Pain et al does not teach the use of PGC's.

Labosky et al teach that murine PGC's cultured in SCF, LIF, and bFGF allows the un-differentation of PGC's into EG cell lines, by a mechanism which has yet to be discovered, and wherein the only difference known to exist between EG's and ES's is the pattern of DNA methylation within region 2 of the Igf2r gene. Labosky et al also teach that both EG's and ES's differentiate in vitro and in vivo, and both EG cells and ES cells can differentiate into functional sperm and thereby transmit their genome through the germline (pg 3201, col 1, para 3).

In light of Pain and Labosky et al, it would have been obvious to one of ordinary skill in the art to culture avian, such as chicken, PGC's in a culture medium comprising 10ng/ml bFGF, 20 ng/ml h-IGF-1, 1% vol/vol avian-SCF, and 1% vol/vol h-LIF. One would be motivated to do this to generate PGC's that could be maintained in culture long-term for facilitating their use in generating chimeras. There would be a reasonable expectation of success because the same culture medium conducted on chicken ES's resulted in at least 35 passages, 160 day utility, and successful germline transmission, therefore a substitution of EG's for ES's would be expected to produce the same results (Labosky et al, pg 3201, col 1, para 3).

Claim 24 is free of the prior art of record and is allowable.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Carrie Stroup whose telephone number is (703) 306-5439. The examiner can normally be reached on Monday through Friday from 8:30 AM to 6:00 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jasmine Chambers, can be reached at (703) 308-2035. The fax phone number for this Group is (703) 308-0294.

Carrie Stroup

BRUCE R. CAMPELL
PRIMARY EXAMINER
GROUP 1800